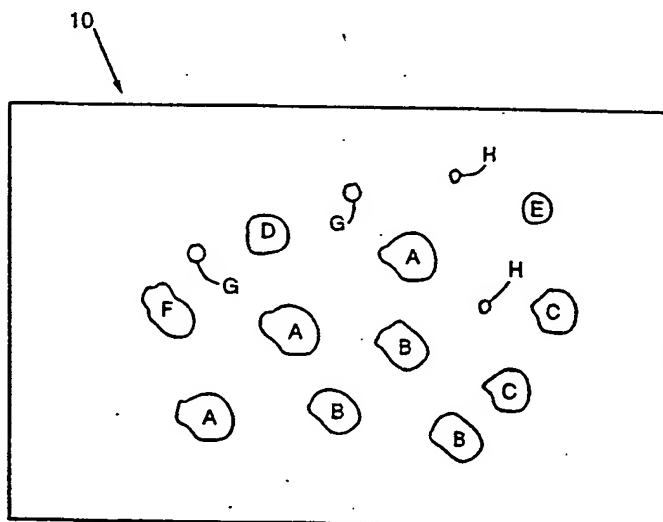




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: <b>PCT/US90/04446</b> (22) International Filing Date: <b>8 August 1990 (08.08.90)</b> (30) Priority data: <b>392,057</b> <b>10 August 1989 (10.08.89)</b> <b>US</b> (71) Applicant: <b>INTERNATIONAL REMOTE IMAGING SYSTEMS, INC. [US/US]; 9162 Eton Avenue, Chatsworth, CA 91311-5805 (US).</b> (72) Inventor: <b>KASDAN, Harvey, Lee ; 5414 Sunnyslope Avenue, Van Nuys, CA 91401 (US).</b> (74) Agents: <b>YIN, Ronald, L. et al.; Limbach, Limbach &amp; Sutton, 2001 Ferry Building, San Francisco, CA 94111 (US).</b>		(81) Designated States: <b>AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).</b>  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>

(54) Title: **A METHOD OF DIFFERENTIATING PARTICLES BASED UPON A DYNAMICALLY CHANGING THRESHOLD**



## (57) Abstract

A first parameter is measured for each of the plurality of different particles. One of the type of particles (A) is identified based upon the first parameter measured. Once the one type of particle is identified, a second parameter, different from the first parameter, is then measured for the one type of particle. The other types of particles (B) in the field of view can be identified based upon the second parameter associated with the one type of particle which has been identified. In one example, color is first used to differentiate various different particles in the field of view and to identify one particular type of particle. Thereafter, a morphological parameter, such as size, associated with the one type of particle identified is measured. The measured morphological character associated with the one type of particle identified is used as a threshold to differentiate other particles in the field of view based upon the second morphological parameter. Similarly, a morphological characteristic can first be used to differentiate one type of particle. A second spectral parameter, such as color, can be measured for the one type of identified particle and can be used to differentiate other particles.

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A METHOD OF DIFFERENTIATING PARTICLES BASED UPON A  
DYNAMICALLY CHANGING THRESHOLD

Technical Field

5 The present invention relates to a method of  
differentiating particles from a plurality of  
particles, based upon a threshold which is  
dynamically changing. More particularly, the present  
invention relates to a method of differentiating  
10 particles wherein the threshold to differentiate the  
particles is determined by a measurement using yet  
another parameter.

Background Of The Invention

15 Methods to identify different particles in a  
field of view are well-known in the art. See, for  
example, U.S. Patent No. 4,175,860. Typically, the  
particles that are under examination are biological  
particles, and it is desired to automatically  
identify the different particles under examination.

20 One of the problems faced by the prior art is  
that various parameters (such as color) associated  
with each particle can vary from one sample to  
another sample. This variation can be caused, for  
example, by different stains used in the preparation  
of different samples or, by the age of the samples.  
25 Thus, the same type of particle appearing in  
different images of view may exhibit different  
parameters.

30 One solution that has been offered by the prior  
art is to add a normalizing substance which has a  
predetermined, a priori detection level. Thus, for  
example, calibrator particles, as disclosed in U.S.  
Patent No. 4,338,024 can be added to a sample. Based

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upon the a priori knowledge of the color of the beads, if the color of the particle under examination exceeds that threshold, then that particle is identified as of a particular type. Clearly, such a normalizing substance is inadequate because the parameter of the particle can vary from sample to sample (due to the factors of different stain and aging as discussed above), whereas the parameter of the normalizing substance does not.

In another example of the prior art solution, the DNA of all the cells are measured. A normalizing value which is manually selected for the particular sample lot for that experiment is selected and that threshold is then used to identify the particles whose DNA exceeds that threshold or is below the threshold. With this approach, while the threshold can vary, it requires manual interpretation and intervention to determine the particular threshold adequate for each of these experiments.

#### Summary Of The Invention

In the present invention, a method of identifying a plurality of different particles in a field of view is disclosed. The method comprises the steps of measuring a first parameter for each one of the plurality of different particles. One of the particles is identified based upon the first parameter measured for the one particle. A second parameter, different from the first parameter associated with the one particle, is then measured for the one particle identified. The other particles in the field of view are identified based upon the second parameter associated with the one particle that has been identified.

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Brief Description Of The Drawings

Fig. 1 is a schematic diagram of an image of a view with a plurality of different types of particles shown.

5      Detailed Description Of The Drawings

Referring to Fig. 1, there is shown an image 10 of a plurality of particles in a field of view. Although the method of the present invention will be described with respect to biological particles in a field of view, it can be appreciated that the method of the present invention can be practiced with any type of particles or with any type of members in a group of members.

As shown in Fig. 1, a plurality of different types of particles (A...H) are shown in the image 10. Thus, there are three particles of type A, three particles of type B, two particles of type C ... and two particles of type H. However, when an image 10 of a plurality of particles is presented, the type of particles is not a priori known. Further, statistical variations in certain parameters of a type of particle may exist. For example, a type A particle may be "larger" in size than the particles of type B and type C. However, not all particles of Type A may be of the same size. The method of the present invention distinguishes the type of particles and identifies them in the image 10 in the field of view.

As used herein, the term "identify" means to determine the label or identifier which is attached to a particular particle or group of particles. Further the term also includes the characteristics, e.g. statistical variations, of the identified group

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of particles. The term "distinguish" or "differentiate" means to separate a group of particles into two or more subpopulation groups based upon one or more parameters. The separation, however, does not necessarily involve identification of the subpopulation groups, i.e. determination of the label for each of the subpopulation groups.

In the method of the present invention, a first parameter is measured for each one of the plurality of particles in the image 10. The first parameter can be a parameter such as color.

Based upon the color intensity of the various particles, one of the type of particles, such as particle A, is identified. Thereafter, a second parameter, different from the first parameter, is measured for the particles which have been identified; namely, particles A. The second parameter can be a morphological parameter; such as size, which is different from spectral parameter such as color. Thus, the particles A identified are then measured for size.

Finally, the other particles in the field of view 10 are identified, based upon the second parameter. The second parameter is measured for each of the other particles. The measurement of the second parameter (for each of the other particles, other than particles identified as being of type A) is compared to the measurement of the parameter determined for the particles identified as being of type A. Based upon this comparison, the other particles are then identified. Thus, for example, the morphological characteristic of size for all the other particles is then compared to the size of particle type A, and is identified based upon this

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comparison. If the size of a particle exceeds that of the size for particles of type A, then the particle is identified as being of a certain type, such as type B.

5           It should be noted while the method has been described with regard to the first parameter, which is measured as being a spectral parameter and the second parameter as being a morphological parameter, the method of the present invention can also be used  
10       where the first parameter is a morphological parameter and wherein the second parameter is a spectral parameter.

          The method of the present invention will now be described with specific reference to the following  
15       examples:

EXAMPLE 1

          In a biological sample containing lymphocytes, monocytes and neutrophils, it is desired to separately identify all of these different types of  
20       particles. However, the difference between monocytes and neutrophils cannot be distinguished based upon color because they differ from sample to sample. Thus, in the method of the present invention, the size of all the different particles is first  
25       measured. Lymphocytes are then distinguished from monocytes and neutrophils by the size of the Lymphocyte particles. If the size of the particles is less than approximately  $50 \mu\text{m}^2$  ( $200 .5 \times .5 \mu\text{m}$  pixel), for example, then the particles are deemed to be  
30       lymphocytes, whereas all other particles contain both monocytes (if any) and neutrophils (if any).

          Thereafter, the color of the lymphocytes so identified is measured. The average color of all the lymphocytes identified is measured. The color

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measured for the identified lymphocytes is then used as a threshold to differentiate monocytes from neutrophils. If the color of a particular particle is greater than the average color of the lymphocytes, then the particle is a neutrophil. If the color of the particle under examination is less than the average color of the identified lymphocytes, then the particle under examination is a monocyte. In this manner, monocytes and neutrophils can be distinguished from one another and identified based upon the average color of lymphocytes in the same sample in which the monocytes and the neutrophils exist.

EXAMPLE 2

A plurality of different types of particles including basophils are shown in an image in a field of view. The basophils are identified by color from all the other types of particles by conventional means or by the use of zeroth green quantile for B021 stained cells. For a description of quantile measurement, see co-pending U.S. application 357,324, filed on May 25, 1989 and assigned to the same assignee as the present invention. That co-pending application is incorporated herein by reference. Thereafter, the area of the identified basophils is measured in pixels and the average area of all basophils thus identified is computed. From the average area of the measured basophils, the pixel size can be determined, assuming a nominal basophil area of about  $42 \mu\text{m}^2$ . From the pixel size determined, the other particles (such as neutrophils, eosinophils and monocytes) can be distinguished based upon this pixel size by computing all particle areas and assigning all particles with areas greater than  $50 \mu\text{m}^2$ .



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to the neutrophil, eosinophil and monocyte category. Alternatively, this decision may be based on the area of a particle relative to the basophil average.

EXAMPLE 3

5           A plurality of different types of particles including known cancer cells and cells that are potentially cancerous are in the imaged field of view. The plurality of different types of particles are subjected to reaction with an antibody which has  
10           a color tag attached thereto. The antibody would attach itself to the known cancer cells. Those known cancer cells would have a particular color, depending on the color tag attached to the antibody. The colored cells are then located and the ploidy of the  
15           known cancer cells are measured. The ploidy of the subpopulation of known cancer cells would have a statistical distribution. Based upon this measured ploidy, the potentially cancerous cells are differentiated from the healthy cells. That is, the  
20           ploidy of the potentially cancerous cells and of the healthy cells are also measured. If the ploidy of a measured unknown cell is approximately on the same order (i.e. within the statistical distribution) as the ploidy of the known cancer cells, then that cell  
25           is potentially cancerous. On the other hand, if the ploidy of a measured unknown cell is far less than the ploidy (i.e. outside of the statistical distribution) of the measured known cancer cells, then the unknown cell is a healthy cell. In this  
30           manner, healthy cells can be distinguished from potentially cancerous cells.

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EXAMPLE 4

A gene probe for human papilloma virus (HPV) is hybridized to a plurality of different cells. The gene probe has an enzyme label attached thereto. Upon introduction of substrate material, the substrate is converted by the enzyme and produces a local colored deposit within the cell. The ploidy of the HPV population (identified by the presence of the colored deposits) may be measured and compared to the ploidy of the non-HPV population (identified by the absence of colored deposits) to determine the degree to which the HPV population has changed from normal.

The theory of operation of the method of the present invention is as follows. The invention is of a method which determines a dynamically changeable normalization threshold from which other particles can be differentiated. Unlike the prior art where the threshold is fixed or is a priori known, the method of the present invention recognizes that the threshold for each sample would differ depending on factors such as age and color stain which vary from sample to sample. Thus, the method of the present invention overcomes the problem of experimental variability; therefore, the same method can be used to differentiate cells in different experiments where the experiments of the different samples are subject to different variables.

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WHAT IS CLAIMED IS:

1. A method of identifying a plurality of different types of particles in a field of view, comprising the steps of:

- 5           a) measuring a first parameter for each of said plurality of different particles;
- b) identifying one of said type of particle based upon the first parameter measured;
- 10          c) measuring a second parameter different from said first parameter, for each of said plurality of different particles; and
- d) identifying other particles in said field of view based upon said second parameter associated with said one type of particle identified.
- 15

2. The method of Claim 1, wherein said first parameter is a morphological parameter and wherein said second parameter is a spectral parameter.

20          3. The method of Claim 1, wherein said first parameter is a spectral parameter and wherein said second parameter is a morphological parameter.

          4. The method of Claim 1, wherein said identifying step of (d) further comprising:

25           measuring the second parameter of other particles in said field of view; and

          using the measured second parameter associated with said one particle to identify other particles in said field of view.

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5. A method of differentiating a first type of cell in a plurality of fields of view where the first type of cell in different fields of view differ in a first detectable parameter, wherein the same method is used to differentiate the first type of cell from other cells in each field of view, said method comprising the steps of:

- a) selecting a field of view;
- b) measuring a second parameter of each of said cells in said selected field of view;
- c) identifying one of said cells, other than the first cell, based upon said second parameter measured;
- d) measuring said first detectable parameter, different from said second parameter, associated with said identified one cell; and
- e) differentiating said first cell from other cells based upon the first parameter measured for said identified one cell.

6. The method of Claim 5, wherein said first parameter is a morphological parameter, and wherein said second parameter is a spectral parameter.

7. The method of Claim 5, wherein said first parameter is a spectral parameter, and wherein said second parameter is a morphological parameter.

8. The method of Claim 5, wherein said differentiating step of (e) further comprising:  
measuring the first parameter of the other cells in the field of view;  
comparing the first parameter of each cell measured to the first parameter of said

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identified one cell and differentiating said first cell from said other cells based upon said comparing.

- 5           9.   A method of identifying a first plurality of members (hereinafter: "a first sub-population") from a group of members, comprising the steps of:
- measuring a first characteristic of each members of said group;
- 10            identifying a second plurality of members (hereinafter: "a second sub-population") from said group based upon said first characteristic measured;
- measuring a second characteristic, different from said first characteristic, of
- 15            each member of said group; and
- identifying the members of said first sub-population based upon the measured second characteristic of each member of said second sub-population.

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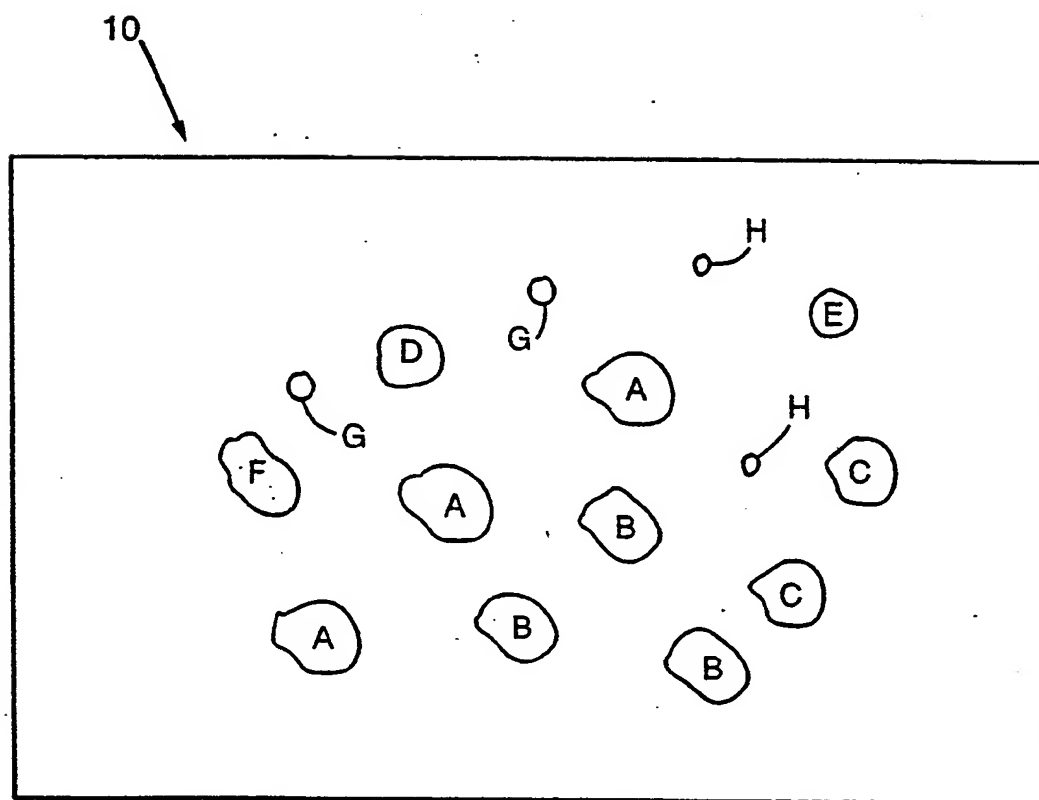


FIG. 1

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US90/04446

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) \*

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5) : G06K 9/00

U.S. Cl : 382/6

## II. FIELDS SEARCHED

Minimum Documentation Searched \*

Classification System

Classification Symbols

U.S. : 356/39; 382/1,6,17,25

Documentation Searched other than Minimum Documentation  
to the Extent that such Documents are Included in the Fields Searched \*

## III. DOCUMENTS CONSIDERED TO BE RELEVANT \*\*

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No. **
X	US,A 4,338,024 (BOLZ ET AL) 06 July 1982 See column 1, 65-69; Column 2, lines 1-11; column 4, lines 67-69.	1-9
A	US,A 4,665,553 (GERSHMAN ET AL) 12 May 1987 See column 3, lines 35-44.	1-9
A	US,A 4,661,913 (WU ET AL) 28 April 1987 See column 4, lines 34-41.	1-9
A	US,A 4,097,845 (BACUS) 27 June 1978 See column 1, lines 58-69; column 4, lines 1-4.	1-9

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## IV. CERTIFICATION

Date of the Actual Completion of the International Search \*

10 SEPTEMBER 1990

Date of Mailing of this International Search Report \*

09 JAN 1991

International Searching Authority \*

ISA/US

Signature of Authorized Official **NGUYEN NGOC-HO**  
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